allogenic primary mixed lymphocytes reaction (MLR) carried out in 96-well microtiter plates by adding different numbers of [The] monocyte-derived antigen-presenting cells to allogenic T cells purified from buffy coats and after 5 days incubation at 37°C, and cell proliferation being assessed by a calorimetric method.--

E4 cont

Claim 65, line 3, change "macrophages" to --MD-APCs--.

Claim 66, line 1, change "The" to --A--.

Add the following new claim:

E5

--87. The process of claim 69, wherein the culture medium contains IL.13.--

R E M A R K S

The claims are further amended herewith, in a manner that is believed to place this application in condition for allowance at the time of the next Official Action.

Two issues were raised in the outstanding Official Action, namely, the repeated rejection of the claims as allegedly being based on a non-enabling disclosure, and a new prior art rejection based on a newly-cited reference. In this latter connection, applicants note with appreciation that the outstanding Official Action was made non-final.

The rejection of claims 48, 52, 56, 57, 59, 60, 62-66, 75, 76, 80 and 81, under the first paragraph of 35 USC §112, as allegedly being based on a non-enabling disclosure, is respectfully traversed, based on the following discussion and evidence.

Literature teaching monoclonal antibodies used for detection and directly coupled to fluorochromes, includes BOYER et al., *Exp. Hematol.*, **27**, 751-761, 1999, and GOXE et al., *Immunological Investigations*, **29(3)**, 319-336, 2000.

The fluorochrome used for each surface marker tested is set forth in the following table:

Surface marker	Isotype of the AcM	Clone and origin	Fluorochrome used
CD14	IgG2a	RMO52 (Beckman Coulter)	PC5 (peridinin chlorophylle 5)
CD64	IgG1	22 (Beckman Coulter)	FITC (FluorolsoThioCyanate)
CD40	IgG1	MAB89 (Beckman Coulter)	PE (PhycoErythrine)
CD80	IgG1	MAB104 (Beckman Coulter)	PE
CD83	IgG2b	HB15a (Beckman Coulter)	PC5
CD86	IgG1	23.31-FUN1 (Pharmingen)	FITC
HLA ABC	IgG1	B9.12.1 (Beckman Coulter)	FITC
HLA DR	IgG2bk	B8.12.2 (Beckman Coulter)	FITC
CD1a	IgG1	BL6 (Beckman Coulter)	PE

The level of intensity of the desired cells versus the intensity of a control is shown on the attached Figure A.

The level of intensity of the desired cells is indicated as unitless Mean Fluorescence Intensity (MFI) and represented on a logarithmic scale. The attached Figure A represents the signal obtained for the detection of HLA DR on

the surface of monocyte-derived cells and the control is indicated in black.

As a control, the non-specific binding on the cells of the fluorochrome labelled antibodies IgG1-FITC, IgG1-PE, IgG1-PC5 and IgG2a-FITC is evaluated. Fluorochrome labelled antibodies of different isotypes and which have no specific interactions with the cells are incubated with the cells. Thus, there is a control for each type of fluorochrome and for each isotype. The intensities of the controls are comprised between 0 and 20.

The Official Action correctly notes that the fluorochrome used is important in the detection of the antigens, and the level of the intensity is different from one fluorochrome labelled antibody to another for the detection of the same cellular antigen. The above discussion and data illustrate, however, that the invention as claimed can be practiced without undue experimentation.

As to the units used for the measurement of Mean Intensity Fluorescence, according to FACS™ Academy, from Becton Dickinson Immunocytometry Systems, Computer Based Training, Volume I "Flow Cytometry and Immunology Basics", basic applications, "flow cytometer detects differences in size, relative granularity and fluorescence, if any, associated with particles. A flow cytometer is not designated to give calibrated measurements unless an external calibrator is used, but instead to detect relative differences in these parameters. That is why a flow cytometer does not provide data in

terms of unit measurements, as a cell counter would." "A beam of laser light is projected across the cells. At the same time, laser light hits the particle, any fluorescence present in or on the particle will fluoresce. Once excited, the intensity of fluorescence signal emitted should be proportional to the amount of the fluorescence compound in the particle." Thus, Mean Fluorescence Intensity (MFI) indicates relative fluorescence intensities for each fluorochrome in a cell sample.

In light of the above discussion, it is believed to be apparent that the rejection of various of the pending claims, as allegedly being based on a non-enabling disclosure, may now be withdrawn. Such action is respectfully requested.

The prior art rejection applied at page 5 of the Official Action contends that all of the pending claims are anticipated, or, alternatively, would have been obvious, based on the disclosure of UNANUE ("Macrophages, Antigen-Presenting Cells, and the Phenomena of Antigen Handling and Presentation", Raven Press Ltd., 1989, pages 95-115). That rejection is also respectfully traversed, for the following reasons.

The UNANUE reference relates to antigen-presenting cells (APC) and more particularly to macrophages. The macrophages, which are mentioned in this reference, are derived from monocytes and this differentiation takes place in tissues (page 96, left column "origin and distribution").

It further teaches that an immunological function of the macrophage is to present antigen in context of class II

MHC. The amounts of class II MHC on the macrophage membrane is key for this cell to function as an APC. But the level of class II MHC varies greatly depending on the tissue.

Macrophage in tissues have particular characteristics: they vary in their extent of surface receptors and expression of class II MHC molecules. Even with a tissue, the macrophage population shows evidence of compartmentalization. For example, the liver macrophages express high levels (> 75%) of class II MHC proteins (page 97, right column) whereas the peritoneal exudate macrophages on average express low amounts of class II molecules (about 10% of the cells) (page 98, top left column).

The present invention concerns monocyte-derived antigen-presenting cells which have phagocytic capacity and which also have capacity for MHC class I (MHC-I) and MHC class II (MHC-II) antigen presentation.

The presentation of MHC class I molecules is not mentioned in the UNANUE reference. However, the ability of MD-APCs of the invention to stimulate CD8-T cells through antigen presentation by MHC class I molecules could not be envisioned after reading this article.

The macrophages according to the invention are derived from the *in vitro* differentiation of blood monocytes, whereas the macrophages, that are mentioned in the UNANUE reference result from the differentiation of monocytes in various tissues. Thus, the tissular macrophages and the MD-APCs according to the invention differ in origin.

Another characteristic of the present invention is that the MD-APCs of the invention form a relatively homogenous population as regards their expression of MHC class II molecules, whereas the UNANUE tissular macrophages show different levels of class II MHC molecules, as mentioned above.

UNANUE also deals with dendritic cells (page 102, left column). As for macrophages, the article describes tissular DCs (from skin, lymphatics, spleen and lymphoid organs, in particular) which is different from the blood-derived cells of the invention. UNANUE cites the "limited evidence of phagocytosis" which is contrary to the cells according to the invention.

Furthermore, at the time of the publication of this article, dendritic cells were not known to derive from monocytes. Thus, UNANUE's article does ont suggest MD-DC according to the invention.

But perhaps the most important characteristic of the present invention, as presently appreciated, is that it concerns a previously unknown "hybrid" type of monocyte-derived antigen-presenting cells that presents some of the characteristics of the macrophage cell-line (e.g. phagocytosis) but other characteristics of dendritic cells (e.g. antigen-processing and antigen-presenting capability).

As is known in the art, macrophages can efficiently interiorize, process and present exogenous antigens to lymphocytes in the context of MHC class I and class II, but they

appear less potent than dendritic cells in the stimulation of lymphocytes. Actually, the recognized limitation of macrophages is that they are not very potent in the priming of a specific immune response against a specific exogenous antigen by stimulation of MHC class I restricted cytotoxic T lymphocytes (CD8+). However, they are more efficient in presenting antigens in the context of MHC class II molecules to T helper lymphocytes (CD4+). These macrophages have the potential to process and present soluble antigens by the exogenous pathway of phagocytes but high concentrations of proteins or antigens are required.

It is also well known in the art that the dendritic cells are the most potent cells for the stimulation of primary T-lymphocyte immune responses. The classical dendritic cells are technically difficult to obtain and they are poorly phagocytozing. In contrast to macrophages, they do not express or have only a dim expression of CD14 and CD64.

In conclusion, the present invention relates to very potent APCs derived from human blood monocytes, which have the following typical features:

- a high phagocytosis activity,
- a high digestive activity,
- a high intensity of antigen presentation (MHC class I and class II),
- an efficient stimulation of T lymphoctyes in MLR reaction.

The APCs of the invention are capable of exhibiting both types of immunological responses to foreign invaders, while displaying a combination of surface antigens characteristic to some extent of macrophages and to some extent of dendritic cells.

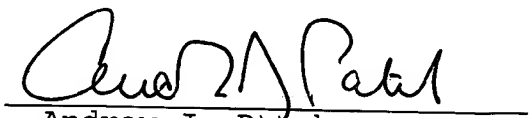
In order that the present claims better reflect the arguments articulated above, the independent claims 44 and 55 now specify MD-APCs which are not tissue macrophages. Given that this aspect of the invention is well-supported by the specification as filed, the language is not objectionable merely by virtue of its being a "negative limitation." See MPEP §2173.05(i).

In light of the present amendments and the above discussion, therefore, it is believed that the rejection based on the UNANUE reference should now be withdrawn.

In view of the present amendment and the foregoing remarks, therefore, it is believed that this application is now in condition for allowance, with claims 44-46, 75, 76, 80, 81 and 87, as amended. Allowance and passage to issue on that basis are accordingly respectfully requested.

Respectfully submitted,

YOUNG & THOMPSON

By 

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November 17, 2000

Figure A

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